R 74-90

United States Patent Office 3,817,838 Patented June 18, 1974

3,817,838

METHOD OF DETECTING AND MEASURING LIPASE ACTIVITY

Norman E. Harris, Waltham, Mass., and Elwyn T. Reese, Caracas, Venezuela, assignors to the United States of America as represented by the Secretary of the Army

No Drawing. Filed Mar. 1, 1972, Ser. No. 230,942
Int. Cl. G01n 31/14
U.S. Cl. 195—103.5 R 5 Claims

ABSTRACT OF THE DISCLOSURE

Lipase activity is detected and measured by incubating an aqueous solution extracted from a suspected fat-containing material with a solution of polyoxyethylene (20) sorbitan monolaurate and assaying the incubated solution to measure the reduction in ester groups of the polyoxyethylene (20) sorbitan monolaurate resulting from lipase induced deesterification.

BACKGROUND OF THE INVENTION

This invention relates to a method for the detection and measurement of lipase activity which utilizes a novel test substrate.

Fats and oils, upon storage, and in the presence of certain enzymes and moisture, undergo hydrolytic decomposition to form free fatty acids. Such free fatty acids combine with available sodium or potassium ions to form fatty acid salts, which are characterized as soaps. In foodstuffs containing fats and oils, hydrolytic decomposition or lipolysis is an undesirable phenomenon producing unwanted soapy or sour flavors in the product. This hydrolytic decomposition does not occur, however, unless some type of catalyst is present such as lipase enzymes or molds (fungi) which normally produce and release lipase enzymes during growth. If the fat or oil containing foodstuff is produced under such conditions as to be free of viable lipase-producing fungal microorganisms or active lipase materials, then hydrolytic decomposition of the fats or oils will not normally occur on storage. While treatment with moist heat will inactivate enzymes or microbes capable of producing such enzymes, a simple procedure is necessary to determine if lipases are present and if lipase-producers are totally inactivated during processing. A procedure, described by Arnulf Purr in an article entitled "Test Paper for the Detection of Esterases in Animal and Plant Tissues and in Microorganisms" appearing in Rev. Inter. Choc., 17, 567–571, December 1962, is based upon a test for the presence of non-specific esterases, i.e., enzymes capable of splitting esters of base fatty acids, e.g., acetates, and butyrates. Such enzymes are said to be commonly encountered along with lipase enzymes in animal and plant tissues and therefore a test for the former is said to indicate the presence of the latter. In this test procedure, free indoxyl is split from indoxyl acetate by esterases in the presence of moisture (pH 7.2–7.5) with the free indoxyl being oxydized by atmospheric oxygen to form an insoluble blue pigment. This test, however, has been found to be too specific in that enzymes that attack and hydrolyze the acid chain would not necessarily hydrolyze the longer acid chains of 12 or more carbon atoms, such as found in lauric oils or fats. Correspondingly, enzymes that would hydrolyze the 12 or more carbon chain fatty acid esters would not necessarily hydrolyze the shorter acetate chain. Therefore, this test procedure has not proved to be a reliable indication of the presence or absence of lipase activity.

SUMMARY

This invention is concerned with a novel procedure to test for the presence of lipase activity in fat-containing materials. This procedure makes use of a novel test substrate, polyoxyethylene (20) sorbitan monolaurate, which is de-esterified by lipase enzymes capable of de-esterifying a medium-chain fatty acid ester. A solution of the test substrate is combined with an aqueous extract of the material being analyzed and then incubated. Any lipase enzyme present will, under the incubation conditions, de-esterify the test substrate. Reduction in ester content of the incubated mixture is determined colorimetrically by hydroxylamine assay.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Lipase activity is the ability of certain enzymes (lipases) to hydrolyze or de-esterify esters of medium-chain fatty acids. Lipases may be found naturally in animal or vegetable cells and are produced during the growth of certain fungal microorganisms. For hydrolytic decomposition of fat to occur, there must be present, in addition to the lipase enzymes, water in excess of 2% by weight. The fatty acids liberated as a result of the lipase induced de-esterification combine with available sodium and potassium ions to form soap-like compounds having noticeable "soapy" or "sour" taste properties.

The novel substrate used herein is polyoxyethylene (20) sorbitan monolaurate (POESM hereinafter) which is a mixture of laurate partial esters of sorbitol and anhydrides condensed with approximately 20 moles of ethyleneoxide for each mole or sorbitol and its mono- and dianhydrides. This material is soluble in water, ethyl alcohol, ethyl acetate, methanol and dioxane, but insoluble in mineral oil and in mineral spirits. The POESM is further described and characterized at pages 525 and 526 of Food Chemical Codex, first edition, published by the National Academy of Sciences, National Research Council, 1966, and is commercially available as Tween 20, a product of Atlas Chemical Industries, Inc., Wilmington, Del.

The procedure of this invention which tests for the presence of lipase activity in a fat-containing material requires that the fat-containing material be mixed with and suspended in water so as to extract any water-soluble lipase enzymes that may be present therein. The fat-containing suspension is then filtered and the aqueous filtrate is reserved. A standard solution of the test substrate, POESM, is prepared by adding 0.5 ml. of the substrate to water buffered to a pH of 7.0 or 4.0 at 2.5% volume/volume. The aqueous filtrate is added to the substrate solution and incubated under conditions which will allow de-esterification to occur. It is preferred that the incubation occur at temperatures of from 35 to 55° C. and for time periods ranging from 1 to 4 hours. The incubated solution is then assayed to determine loss of ester in the test substrate. The assayed procedure is described in detail by S. Hestrin, in the *J. Biol. Chem.*, 180, 249, 261 (1949) in an article entitled "Determination of Acetyl Content." This procedure is based upon the ability of esters to react with hydroxylamine quantitatively in an aqueous alkaline solution to form a purple-brown color, the intensity of which decreases with increasing substrate de-esterification. The test can be done on either a qualitative or quantitative basis.

Any fat-containing material may be tested for the presence of lipases according to this invention. Fat is defined herein as a medium-chain fatty acid glyceride ester. Medium-chain fatty acid is a saturated or unsaturated aliphatic monocarboxylic acid having a chain length of from 12 to 24 carbon atoms. The enzymatic de-esterifi-

cation of fat is especially significant in foodstuffs, since the off-flavors produced render the product unacceptable or unpalatable. This test procedure, therefore, will be especially desirable in assuring the necessary control of quality of fat-containing foodstuffs. Cocconut and other high lauric oils or fats (triglycerides) such as palm kernel, babasu, tucum or mixtures thereof are highly susceptible to lipase-hydrolysis. Coconut is frequently a component of candy and is likely to be stored for considerable lengths of time at ambient conditions. In order to successfully survive even short term storage under such conditions, it is essential that the coconut be free of lipase activity to prevent hydrolytic decomposition or de-esterification, resulting in a rancid or "soap" flavored product.

The following example illustrates in detail the procedure of this invention.

EXAMPLE I

5 grams of finely ground coconut meat is combined with 25 ml. of an aqueous solution of 0.15 M potassium chloride (pH 6.0). This suspension is agitated for 12 hours by continuous shaking at room temperature. The suspension is filtered and the aqueous filtrate is reserved. The test substrate (POESM) is added to water buffered to a pH of 7.0 at 2.5% volume/volume. 0.5 ml. of the filtrate is added to 0.5 ml. of the substrate solution and the resulting mixture incubated in a water bath at 35° C. for 3 hours. The incubated solution is then assayed for ester content using the hydroxylamine assay procedure of S. Hestrin, to which reference has already been made. In the assay, equal volumes of 0.2 M hydroxylamine are mixed with 3.5 N sodium hydroxide. 1 ml. of the incubated solution and 1 ml. of alkaline hydroxylamine solution are combined. After one minute, 1 ml. of concentrated hydrochloric acid solution (1 volume hydrochloric acid, 2 volumes water) is added, followed by 1 ml. of a ferric chloride solution (0.37 M ferric chloride in 0.1 N hydrochloric acid). The density of purple-brown solution is determined at 540 millimicrons in a Lumetron colorimeter 401A with a blank set of 100% transmission. This same procedure was repeated with nonfat dry milk powder, chocolate liquor, and cocoa. Lipase activity was detected only in the coconut. The percent transmission for the coconut was 31%, for milk powder 8%, for chocolate liquor 8% and for cocoa 5%.

This invention provides a solution to the lipase detection problem in coconut containing foodstuffs such as

candies and can also be used in connection with other types of fat-containing foods which could contain or be contaminated with lipase enzymes, such as intermediate moisture (7 to 12%) foods, dry fat containing food mixed with dry ground spices and certain cereal products.

We claim:

1. A method for detecting lipase activity in a fat-containing food stuff material which consists of the following steps in sequence:
   (a) dispersing and agitating a quantity of said fat-containing material in water to facilitate extraction of water-soluble components,
   (b) filtering the dispersion and reserving the aqueous filtrate,
   (c) combining a solution of polyoxyethylene (20) sorbitan monolaurate with the filtrate,
   (d) holding the mixture of step (c) under incubation conditions for sufficient time to allow de-esterification to occur, and
   (e) assaying the mixture for loss of ester material by means of hydroxylamine assay wherein an aqueous alkaline solution of hydroxylamine is first added to said mixture and then an acidic solution of ferric chloride is added thereto to form a colored solution in the presence of ester material, the density of the color being related to the amount of ester material in the solution.

2. A method according to claim 1 wherein said mixture is incubated at a temperature ranging from 35° to 55° C. and for a time ranging from 1 to 4 hours.

3. A method according to claim 2 wherein said suspension filtrate and said test substrate are buffered to a pH of 7.0 or 4.0.

4. A method according to claim 3 wherein said fat is a glyceride ester of one or more medium-chain fatty acids.

5. A method according to claim 4 wherein said fat material is coconut oil.

References Cited

"Analytical Abstracts," vol. 1, 2781 (1954).
Hestrin: "J. Biol. Chem.," 180, 249–261 (1949).
White et al.: "Prin. of Biochem.," 3rd ed. (1964), pp. 62–63.

ALVIN E. TANENHOLTZ, Primary Examiner